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Discovery of a potent CDK2 inhibitor with a novel binding mode, using virtual screening and initial, structure-guided lead scoping

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Abstract—Virtual screening against a pCDK2/cyclin A crystal structure led to the identification of a potent and novel CDK2 inhibitor, which exhibited an unusual mode of interaction with the kinase binding motif. With the aid of X-ray crystallography and modelling, a medicinal chemistry strategy was implemented to probe the interactions seen in the crystal structure and to establish SAR. A fragment-based approach was also considered but a different, more conventional, binding mode was observed. Compound selectivity against GSK-3β was improved using a rational design strategy, with crystallographic verification of the CDK2 binding mode.

Virtual screening is now recognized as an important tool in the armoury of methods which can be applied to hit identification in the industrial environment.[1–4] One popular approach involves the use of high throughput docking methods to screen catalogues of compound structures, in order to select a subset of molecules for acquisition and assay. A number of successful applications have been reported, including the discovery of ligands targeting the Chk1 kinase[5] and Hsp90.[6] Whilst virtual screening methods can be applied to homology models,[7–10] they are more typically used in conjunction with experimental structural data, particularly X-ray crystallographic data. The availability of crystal structures greatly aids the virtual screening process and also provides important insights into the kinds of changes in protein conformation, which may be significant in determining both likely hit rates, and also the kinds of ligands which may be identified as hits.[11,12]

Kinases comprise one of the most important families of drug targets, accounting for 20–30% of the drug discovery programmes of many companies, second only to GPCRs.[13] They are of particular interest in oncology, as a number of them are involved in the regulation of cell growth and survival. In addition, the CDK2 protein is highly amenable to crystallography, with a large number of crystal structures, both of the monomeric form of the protein and the activated pCDK2/cyclin A complex, being available. In common with some earlier studies,[14–16] this report documents the use of the activated, and possibly more physiologically relevant, complex in a protein structure-guided drug discovery strategy. However, it should be noted that where the same ligand has been crystallized with both the monomeric and activated complexes (unpublished data), there is generally good agreement between the observed binding modes in our hands. Differential packing interactions may affect some ligands, however, as can be seen in the structures with pdb codes 2C6M and 2C6T.[17,18]

CDK2, along with other cyclin dependent kinase (CDK) family homologues, is known to be important in regulating entry into, and progression through, the cell cycle.

Keywords: Virtual screening; Docking; CDK2; Cyclin dependent kinase; X-ray crystallography; Structure-guided drug design; Fragment based.
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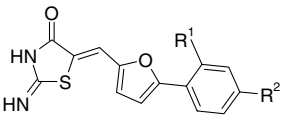
Whilst some selectivity against other kinases is desirable in order to mitigate against unwanted off-target effects, it is not necessary to have absolute selectivity, since a degree of promiscuity may help to circumvent resistance mechanisms. This may be particularly pertinent for CDK2, as recent literature reports[19,20] suggest that there may be a degree of functional degeneracy within the CDK family. In particular, selectivity over CDK1 was not actively sought. For the lead scoping phase of the project, it was decided to monitor selectivity over glycogen synthase kinase-3β (GSK-3β) as the main counter-screen. GSK-3β was chosen as it has a therapeutically antagonistic effect to CDK2, but also exhibits some similarities in its small molecule inhibition profile.[21,22]

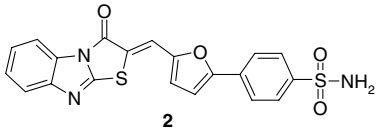
The public domain structure of the activated complex bound to ATP[18] [pdb code-1QMZ [23]] was chosen as a base for the virtual screen, since this is the most physiologically relevant cavity. It was anticipated that knowledge of the other CDK2 cavities, and the glycine loop flexibility, could be leveraged during any subsequent hit-to-lead or lead optimization activities.

A high throughput docking protocol, using rDock,[24] was employed to screen a docking library of 975,000 compounds against the CDK2 ATP binding site. This library was derived from rCat,[25] a diverse compound collection derived from vendor catalogues of commercially available compounds, following filtering to remove compounds containing unwanted chemical functionality and also those with poor calculated physicochemical properties. A two-stage docking protocol was used, with score thresholds set based on experience with this and other kinase targets. Whilst it was well recognized that the majority of kinase inhibitors interact with the protein though hydrogen bonds to the conserved kinase backbone motif (Glu81 and Leu83 for CDK2), no attempt was made to enforce this other than a requirement for the ligand to occupy the pocket in the vicinity of this motif. This region comprises the deepest and most constrained region of the binding site. The aim of the screen was to identify ligands which were as diverse and novel in nature as possible. This decision was partly based on the very competitive nature of the CDK2 area, with its wealth of pre-existing IP, and also partly on a wish to complement other approaches being pursued in-house.[17,26]

Compounds which docked in poses meeting the required score threshold were subjected to further triage. This process included removal of those compounds with <1 μM predicted solubility,[25] those previously screened in-house, those with a poor balance of polar and hydrophobic properties and those flagged by the GP filter algorithm.[27] The solubility calculation, which gives a cross validated $r^2 = 0.81$ on publicly available compounds using a leave out group of 5 method,[25] provided a means of enriching the surviving set with compounds likely to demonstrate a satisfactory level of aqueous solubility. It should be remembered, however, that whilst this high throughput calculation performs very well on large datasets, individual compound properties may not always be well predicted. The remaining set of 18,800 compounds was subjected to diversity analysis,[28] followed by visual inspection of binding modes. A set of 1121 compounds were purchased and assayed, resulting in 38 hits with an IC_{50} value of less than 100 μM. The top concentration in the assay, whilst relatively high, was chosen to maximise the likelihood of obtaining structurally diverse hits that could be crystallized in CDK2, so as to gain information on diverse scaffolds and interactions of interest. The assay results corresponded to a 3.4% hit rate, which compared favourably to the 1.2% hit rate which resulted from a medium throughput screen of 3341 compounds which had been sourced from commercial kinase-biased libraries.[26] Two compounds of similar chemical class, and with sub-micromolar activity against CDK2, were identified (**1**; IC_{50} 0.18 μM and **2**; IC_{50} 0.76 μM).[29] These were the only compounds with $IC_{50} < 10$ μM, with a further 7 compounds having $IC_{50} < 20$ μM. Other analogues of compound **1**, with lower CDK2 potency (**3**; IC_{50} 19 μM and **4**; IC_{50} 48 μM), also appeared in the hit set, giving confidence that there would be directional SAR. This paper reports the initial, structure-guided lead scoping around **1**, which was employed to assess its suitability for progression to lead optimization.



1 R^1 = H, R^2 = SO_2NH_2
3 R^1 = NO_2, R^2 = H
4 R^1 = H, R^2 = CO_2H



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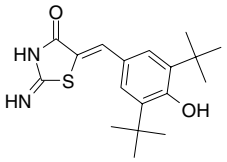
Preliminary exploration around the structure of **1** revealed no prior art with respect to CDK2, although one patent[30] claimed very similar compounds as inhibitors of VEGF, which is also an oncology target. The unusual nature of the 2-imino-thiazolidin-4-one moiety of compound **1** also prompted considerations as to whether the compound would be tolerated in vivo. Literature analysis provided no reports of toxicity, and supporting information was obtained in the form of darbufelone **5**,[31,32] a compound which is reported as having been in phase II clinical trials.



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Screening compound **1** in a cross-kinase panel[29] gave encouraging selectivity data (CDK1 IC_{50} 0.32 μM; GSK-3β IC_{50} 0.23 μM; PDK1 IC_{50} 67 μM; PKA IC_{50} 77 μM; AKT with 54% inhibition at 10 μM; Chk1 with 44% inhibition at 50 μM), confirming that this

compound is unlikely to be a frequent hitter.[33–35] Compound **1** showed no selectivity over GSK-3β, however, so a key aim was to determine if this could be introduced, as well as improving the observed poor aqueous solubility.

Compound **1** can exist as different geometrical isomers and tautomers; it was therefore of interest to determine the nature of the active species. Detailed modelling work was unable to assign the chemical structure unambiguously, with both *E*- and *Z*-isomers having the ability to dock. The bound *Z*-isomer was subsequently ascertained from the unambiguous electron density for **1** in complex with pCDK2/cyclin A. Whilst the virtual screen had suggested an extended pose for **1**, more exhaustive docking, with a solvation correction, suggested a "bent" binding mode to be more favourable, with the central furan being near the kinase motif, the aryl sulfonamide extending towards solvent and the 2-imino-thiazolidin-4-one moiety interacting with the floor of the binding site. Interestingly, for docking into the 1QMZ[23] complex, the solvation correction is needed to produce this binding mode. However, docking into the 1CKP structure,[18,36] chosen as a general purpose monomeric CDK2 cavity which had performed well in limited cross docking experiments, suggested the bent binding mode to be preferred, irrespective of the solvation term.

Analysis of the crystal structure of compound **1** co-complexed with pCDK2/cyclin A clearly showed the bent binding mode to be present, consistent with the modelling, and with the unconventional interaction to the kinase binding motif (Fig. 1).

The crystal structure of **1** bound to the activated form of CDK2 had two complexes in the asymmetric unit, with subtle differences in the ligand interactions observed in each case. The ligand, which has little conformational freedom and therefore a low entropic penalty to binding, has very good steric complementarity with the active site and the molecule is tethered at either end by hydrogen bonding. However, in one of the chains, the ligand sulfonamide group interacts with the backbone NH of Asp86 (2.8 Å between heavy atoms) and also the side chain (3.1 Å), whilst in the other chain the interactions are to the backbone NH of Asp86 (3.1 Å) and the backbone carbonyl of Leu10 (3.3 Å). The structural data suggested that the 4-sulfonamide group was available for elaboration, as long as some of the hydrogen bonding character of the sulfonamide nitrogen was maintained, providing a handle for elaboration to moderate physicochemical properties such as solubility. As shown in Table 1 the methyl sulfonamide (**6**) maintains good potency (CDK2 IC_{50} 120 nM) and **7**, a solubilised variant, has a CDK2 IC_{50} of 570 nM. In contrast, compounds **8** and **9**, in which the sulfonamide NH is replaced, lose significant potency, as does a carboxylic acid variant (**4**), and the amide **10**. This is consistent with the importance of the interactions to this part of the ligand. When interpreting the SAR, however, it should be remembered that the compounds can exist as more than one regioisomer, and the relative populations of these species have not been examined.

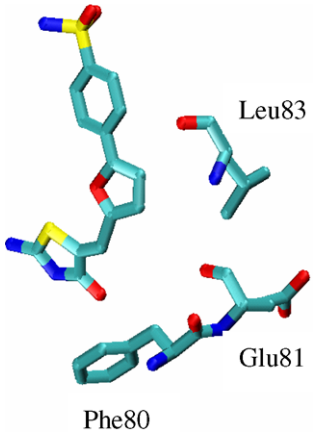


Figure 1. Kinase motif interactions in the crystal structure of compound **1** with pCDK2/cyclin A.[37]

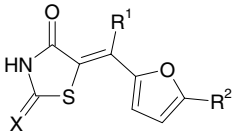
The structural data also suggested that there was scope for elaboration of the 3-position of the phenyl ring, since one edge of the ring is solvent exposed in the active site. This was also tolerated, with a very similar binding mode for **11** being verified by crystallography, although with the bound regioisomer having the trifluoromethyl group packing against the protein rather than facing out into solvent. Compounds **11** and **12** showed reverse selectivity, being highly potent against GSK-3β.

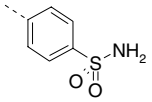
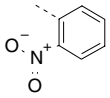
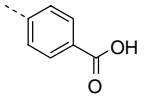
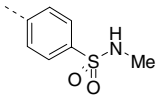
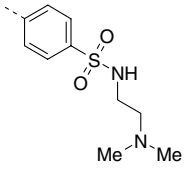
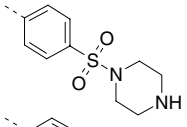
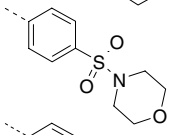
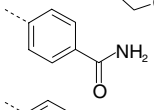
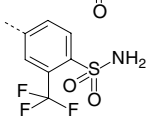
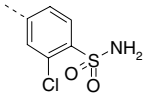
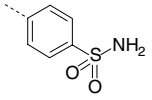
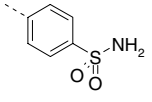
The 2-imino-thiazolidin-4-one moiety of **1** binds in the crystal such that the carbonyl group interacts with a bridging water (3.0–3.5 Å), the ring nitrogen interacts with the backbone NH of Asp145 (3.4–3.5 Å) and very weakly with Lys33 (3.7–4.0 Å) and the imino group in one of the two chains interacts with the Asp145 side chain (3.1 Å). Replacement of this exocyclic nitrogen by oxygen (**14**) is detrimental to activity.

As shown in Table 1, crystal structures are available for the complexes of compounds **1**, **4**, **6**, **11**, **13**, and **14**, with either pCDK2/cyclin A (co-crystals) or monomeric CDK2 (soaks), and these all broadly demonstrate the expected binding modes. The ligands do shift relative to each other in the binding site (by approximately 1 Å), however, and the protein exhibits a high degree of induced fit or malleability around some of the ligands. In each case, it is the *Z*-isomer of the compound that is bound in the crystal structure.

A key aim was to improve selectivity over GSK-3β, and previous work[26] had shown this could be achieved by improving packing against Phe80. Methylation of the double bond provided a means of achieving this, and improved GSK-3β selectivity from 1.4-fold to over 14-fold, as illustrated by compound **13**. The crystal structure of **13** complexed with CDK2 showed a binding mode very similar to that expected, with the methyl group packing against Phe80, but with a degree of induced fit and an improved interaction between the iminothiazolidinone ring and the side chain of Asp145. Unfortunately, all compounds had an HCT116 GI_{50} of >80 μM in an SRB assay, other than compound **9** (HCT116 GI_{50} 40 μM).[38]

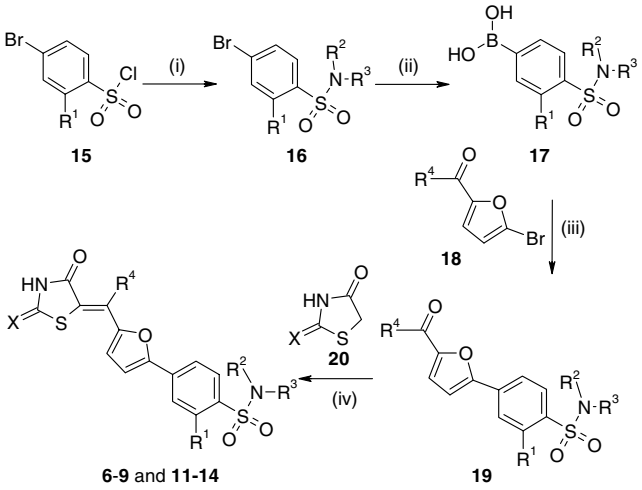
Table 1. Enzyme activity (CDK2 and GSK-3β) and X-ray crystallographic data for compounds **1**, **3**, **4**, and **6**–**14**



Compound	X	R^1	R^2	CDK2 IC_{50} (μM)[a]	GSK-3β IC_{50} (μM)[a]	X-ray structure type	PDB Code	Resolution (Å)
1	NH	H		0.18	0.25	pCDK2/ cyclinA	2uzd	2.8
3	NH	H		19		No		
4	NH	H		47		pCDK2/ cyclinA	2uze	2.4
6	NH	H		0.12	0.08	pCDK2/ cyclinA	2uzb	2.7
7	NH	H		0.57	0.51	No		
8	NH	H		2.9		No		
9	NH	H		150		No		
10	NH	H		9.3		No		
11	NH	H		0.61	0.002	pCDK2/cyclinA	2uzl	2.4
12	NH	H		0.57	0.014	No		
13	NH	Me		0.03	0.43	CDK2 low occup.	2uzn	2.3
14	O	H		27		CDK2	2uzo	2.3

[a] Values are means of at least two determinations and are rounded to two significant figures. For CDK2, [ATP] = 100 μM ($2 \times K_m$) and for GSK3β, [ATP] = 10 μM ($1 \times K_m$).

Compounds **6**–**9** and **11**–**14** were synthesized in accordance with Scheme 1. Sulfonamides of type **16** were prepared from 4-bromobenzenesulfonyl chlorides **15** (R^1 = H, CF_3 or Cl) and the appropriate amine in the presence of pyridine. Subsequent conversion to boronic acids of type **17** was achieved using *n*-butyl lithium and triisopropyl borate. In the case of primary sulfonamides, Boc-protection of **16** ($R^2 = R^3$ = H) was required prior



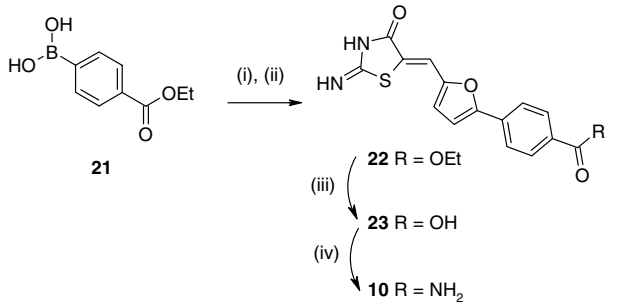
Scheme 1. Reagents and conditions: (i) R^2R^3NH, C_5H_5N, CH_2Cl_2, rt, 1 h, quant.; (ii) (for $R^2 = R^3 = H$) Boc_2O, Et_3N, DMAP; $B(i\text{-}PrO)_3$, *n*-BuLi, THF, −78 °C to rt, 18 h, 40%; (for 2^y and 3^y sulfonamides) $B(i\text{-}PrO)_3$, *n*-BuLi, THF, −78 °C to rt, 18 h, 40%; (iii) (for $R^4 = H$) 5-bromo-2-furaldehyde, THF–H_2O (10:1 v/v), K_2CO_3, Pd(dppf)Cl_2, μ wave, 120 °C, 5 min; (for $R^4 = Me$) 1-(5-bromofuran-2-yl)ethanone, THF–H_2O (10:1 v/v), K_2CO_3, Pd(dppf)Cl_2, μ wave, 140 °C, 5 min, 40–80%; (iv) (for $R^4 = H$, X = NH) 2-imino-thiazolidin-4-one HBr salt, piperidine, PhMe, AcOH, Δ; (for $R^4 = Me$, X = NH) 2-imino-thiazolidin-4-one HBr salt, PhMe, NMP, NH_4OAc, μ wave, 240 °C, 30 min; (for $R^4 = H$, X = O) thiazolidine-2,4-dione, piperidine, PhMe, AcOH, Δ, 5–15%.

to this step, which was then cleaved during the final condensation. Compounds of type **17** were coupled with 5-bromo-2-furaldehyde **18** ($R^4 = H$) in the presence of potassium carbonate and Pd(dppf)Cl_2 to give furans of type **19**. Condensation of 2-iminothiazolidin-4-one or thiazolidine-2,4-dione and **19** ($R^4 = H$) led to compounds **6**–**9**, **11**, **12** and **14**,[31,39] which were purified to ⩾85% by preparative HPLC.

Treatment of 5-bromo-2-furaldehyde **18** ($R^4 = H$) with methyl magnesium bromide in THF, and subsequent oxidation using a suspension of MnO_2 in dichloromethane, gave 1-(5-bromofuran-2-yl)ethanone **18** ($R^4 = Me$) in 79% overall yield, which underwent Suzuki coupling with compounds of type **17** as described above. Condensation of **19** ($R^4 = Me$) with 2-iminothiazolidin-4-one was achieved by microwave irradiation in toluene/NMP in the presence of ammonium acetate, which afforded compound **13**.[31,39]

Compound **10** was prepared as outlined in Scheme 2. The ethyl ester **22** was prepared from boronic acid **21**, in a manner analogous to that for the synthesis of sulfonamide derivatives **6**–**9** and **11**–**14** in Scheme 1. Hydrolysis of **22** to the carboxylic acid **23**, and subsequent treatment with EDCI and NH_4-HOBT, gave the primary carboxamide **10**.

The binding mode of the commercially available fragment, 2-imino-5-[1-pyridin-2-yl-methylidene]-thiazolidin-4-one **24**, was also studied by X-ray crystallography. Figure 2 shows that compound **24** also bound to CDK2, but in a more extended mode, with the 2-imi-



Scheme 2. Reagents and conditions: (i) 5-bromo-2-furaldehyde, THF–H_2O (10:1 v/v), K_2CO_3, Pd(dppf)Cl_2, μ wave, 120 °C, 5 min; (ii) 2-imino-thiazolidin-4-one HBr salt, piperidine, PhMe, AcOH, Δ; (iii) $NaOH_{(aq)}$, THF, μ wave, 100 °C, 500 s, quant.; (iv) EDCI, NH_4-HOBT, DMF, rt, 18 h, 20%.

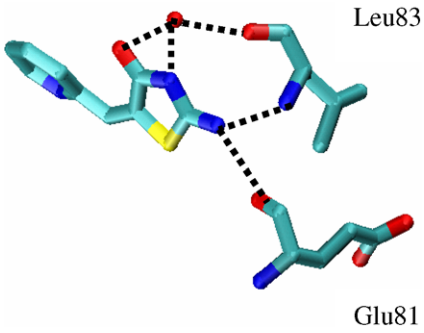
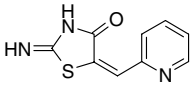


Figure 2. Crystallographically observed binding mode of compound **24**/pCDK2/cyclin A, showing kinase motif interactions.[36] Available from the PDB (ref. 18) under code 2v0d.

no-thiazolidin-4-one moiety interacting with the kinase binding motif, and was the *E*- rather than the *Z*-isomer. The compound binds such that the imino group is able to donate a hydrogen bond to the backbone carbonyl of Glu81 (3.0 Å), and accept one from the backbone NH of Leu83 (2.6 Å). N-3 interacts with Leu83 via a water mediated hydrogen bond (2.4 Å to the water, which is 2.8 Å from the Leu83 carbonyl). The same water also interacts with the ligand carbonyl group (2.7 Å).



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The fragment structure therefore provides new opportunities for structure-guided design, supported by crystallographic data, complementary to the compound series resulting from the virtual screening. This shows the versatility inherent in combining approaches to hit identification. The change in the observed binding mode illustrates the importance of having access to structural data. This can avoid being misled when designing hit to lead or lead optimization strategies for developing series from low molecular weight starting points.

In summary, virtual screening against CDK2 identified a submicromolar inhibitor, with a distinctive binding mode and selectivity over a number of other kinases. A structure-guided lead scoping exercise led to the identification of a 30 nM CDK2 inhibitor **13** with selectivity over GSK-3β of over 14-fold. Crystal structures were obtained for a number of compounds of interest, and provided important validation of the unusual binding mode and support for optimization. It was also observed that the 2-imino-thiazolidin-4-one moiety is able to interact more classically with the kinase binding motif, as was verified by the crystal structure of CDK2 in complex with a smaller ligand, which also contains this functionality.

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